Helq works in parallel to *Brca2* to suppress chromosome instability

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Abstract

Fanconi anemia (FA) is a rare, genetic disorder characterized by genome instability, bone marrow failure, congenital abnormalities, and a high predisposition to cancer. It is caused by mutations in any of at least 16 known genes that act together in a common FA pathway for interstrand crosslink repair, a special type of DNA damage that induces replication fork stalling (see background). Previous research showed that the DNA helicase HELQ works in parallel to FANCC, indicating that it is likely not part of the upstream FA core complex. To determine if HELQ works instead with the downstream FA pathway member BRCA2, we combined our *Helq*^{gt} mutant allele with that of a *Brca2* mutant allele harboring a C-terminal truncation (*Brca2*$^{\Delta 27}$). This allele disrupts important interactions of BRCA2 with FANCD2, making it useful for investigating the role of BRCA2 specifically within the FA pathway. *Helq*$^{gt/gt}$;*Brca2*$^{\Delta 27/\Delta 27}$ double mutant cells showed greatly elevated levels of chromosome instability, including spontaneous micronuclei and 53BP1 nuclear bodies, markers of unresolved replication intermediates. Importantly, this was significantly worse than either of the *Helq*$^{gt/gt}$ or *Brca2*$^{\Delta 27/\Delta 27}$ single mutants, indicating a non-epistatic relationship between *Helq* and *Brca2*. These data suggest that *Helq* may be an effective therapeutic target for correcting chromosome instability in the cells of FA patients.

Background

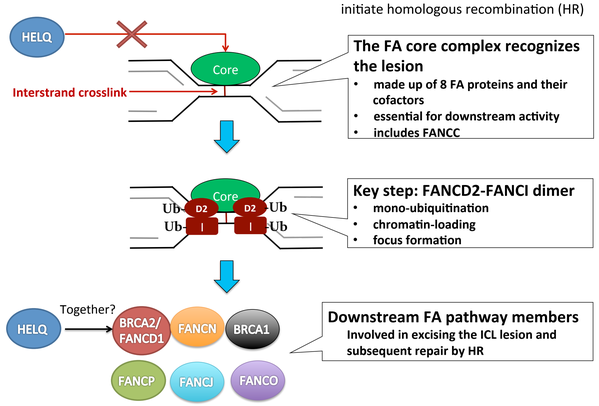
HELQ, BRCA2 and the canonical FA pathway for the repair of interstrand crosslinks (ICLs)

HELQ

- Superfamily 2 DNA helicase
- In FA pathway in lower eukaryotes
- Works in parallel to FANCC
- Not part of the FA core complex
- Important to suppress chromosome instability

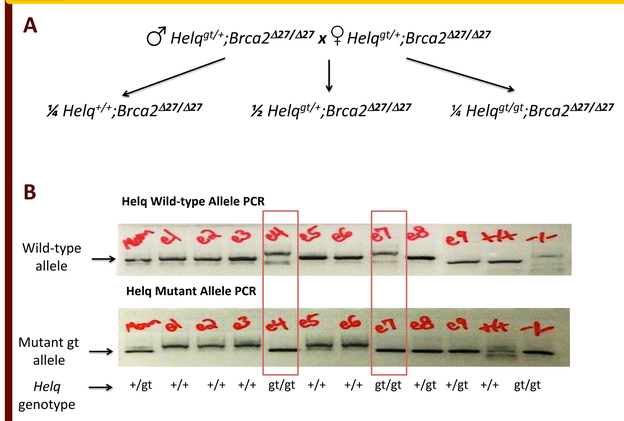
BRCA2/ FANCD1

- Downstream member of the FA pathway
- Deficiency of this gene can lead to FA as well as heightened predisposition to breast/ovarian cancers
- Essential for the recruitment of RAD51 to initiate homologous recombination (HR)



Results

1 Generation of Helq$^{gt/gt}$,Brca2$^{\Delta 27/\Delta 27}$ mutant mice/cells



Note: All mice were found not to carry *Brca2 wild-type allele*

Figure 1. Combining the *Brca2* C-terminal truncation allele (*Brca2*$^{\Delta 27}$) with the *Helq* gene-trap allele (*Helq*^{gt}) to generate *Helq*$^{gt/gt}$;*Brca2*$^{\Delta 27/\Delta 27}$ double mutant mice/cells. A) Mating scheme to derive *Helq*$^{gt/gt}$,*Brca2*$^{\Delta 27/\Delta 27}$ double mutant mice. **B)** *Top: Helq* wild-type allele genotyping. From left to right, mom (*Helq*$^{gt/+}$; *Brca2*$^{\Delta 27/\Delta 27}$, as in 1A), embryos 1, 2, 3, 4, 5, 6, 7, 8, 9, and the positive and negative controls (*Helq*$^{+/+}$ and *Helq*$^{gt/gt}$). *Bottom: Helq* mutant allele genotyping. The red boxes around e4 and e7 indicate that they are *Helq*$^{gt/gt}$;*Brca2*$^{\Delta 27/\Delta 27}$ double mutants.

2 Helq$^{gt/gt}$,Brca2$^{\Delta 27/\Delta 27}$ double mutant mice show a more severe hypogonadism compared to either single mutant

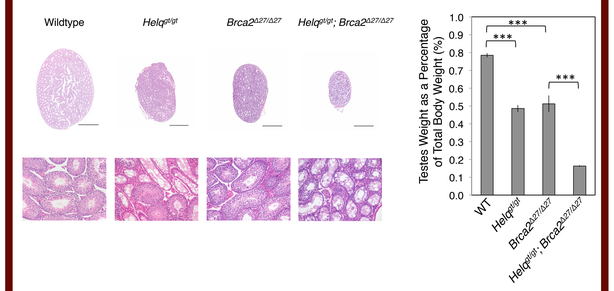


Figure 2. *Brca2* deficiency causes a mild form of hypogonadism, which is not epistatic to *Helq*.
A) Testis cross-sections show a mosaic pattern of empty and filled seminiferous tubules in *Helq*$^{gt/gt}$, *Brca2*$^{\Delta 27/\Delta 27}$ and double mutants at 6 weeks of age. Scale bars are 1500 µm. Enlarged images are 20X. **B)** Relative average testes weights are shown. Asterisks indicate the significance level using a t-test, with *** for p<0.001.

3 Helq$^{gt/gt}$,Brca2$^{\Delta 27/\Delta 27}$ double mutant cells display a drastic increase in replication-associated genome instability

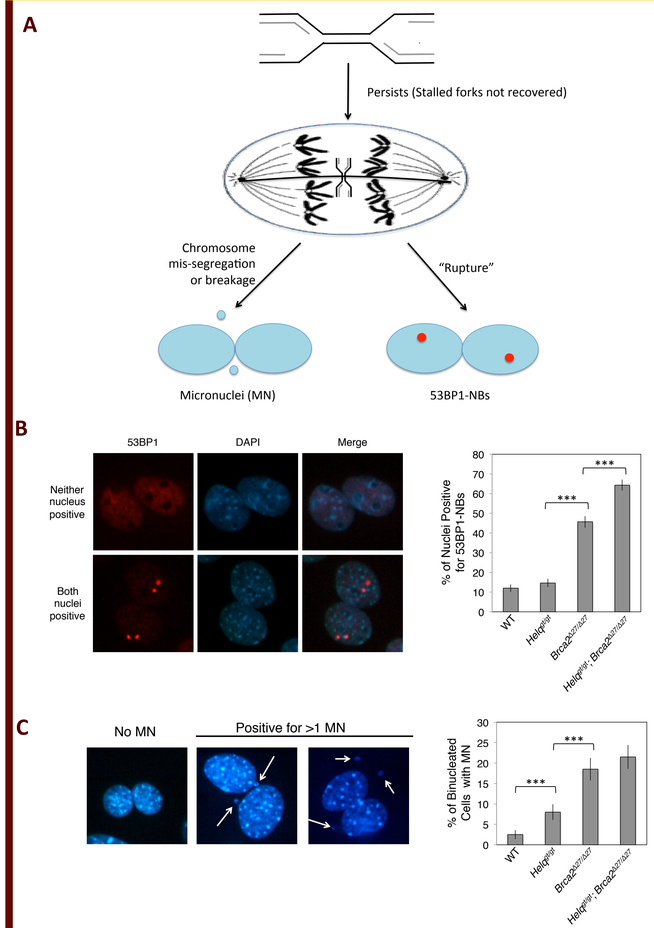


Figure 3. A) Shown is a model depicting how unresolved replication intermediates can result in chromosome mis-segregation/breakage or "rupture" after passage through M phase.
B) *Brca2* suppresses the formation of 53BP1 nuclear bodies (53BP1-NBs) in a manner that is non-epistatic with *Helq*. *Left:* Recently divided G1 phase cells were identified as those that were binucleated following treatment with the cytokinesis-blocking reagent cytochalasin B. Binucleated cells were then stained for 53BP1 (red) and DAPI (blue) and scored for the presence of 53BP1-NBs. Shown are examples of nuclei with and without 53BP1-NBs. *Right:* The average percentages of binucleated cell nuclei positive for 53BP1-NBs are shown for each genotype.
C) *Brca2* suppresses the formation of micronuclei (MN) in a manner that may or may not be epistatic with *Helq*. *Left:* Binucleated cells were scored for the presence or absence of MN. The white arrows show examples of MN. *Right:* The average percentages of binucleated cells positive for MN are shown. Significance (B,C) was determined by χ^2-test, with *** for p<0.001. Error bars (B,C) show the binomial error for the combined data sets.

Summary

Results:

1) *Helq*$^{gt/gt}$;*Brca2*$^{\Delta 27/\Delta 27}$ mice are viable.

2) *Helq* and *Brca2* are non-epistatic for the germ cell maintenance, as double mutant mice exhibit a more severe hypogonadism that either single mutant.

3) *Helq* and *Brca2* also appear to be non-epistatic for the suppression of genomic instability, with *Brca2* having a seemingly more important role.

Conclusion:

HELQ prevents replication-associated genome instability through the recovery of stalled replication forks in a manner parallel to BRCA2

Significance:

1) As *Helq* works in parallel to a downstream FA pathway member, BRCA2, it seems unlikely that HELQ is part of the downstream FA pathway. This also suggests that *Helq* is not an FA gene.

2) As HELQ is critical for maintaining genome stability in the absence of functional BRCA2, it may provide a therapeutic window for all types of cancer involving FA pathway deregulation and BRCA2 deficiency.

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